STRUCTURAL AND FUNCTIONAL MIMIC OF METALLOENZYMES WITH TRANSITION METAL-COMPLEXES

Ph.D. Theses

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INTRODUCTION AND THE AIMS OF THE WORK

It is well known that some metal ions are essential for living organisms while other metals even in small concentration are dangerous for these. Metals and their compounds play important role in many processes of biological systems. Beside many other functions they act as cofactors of enzymes catalyzing hydrolitical and redox processes thus they take part in the synthesis of biomolecules.

Exploration of the working mechanism of metal ions and their compounds can't be effective without the knowlegde of their biospeciation. For these reasons small molecular model compounds are extensively applied to imitate the active sites of biomolecules. Coordination chemical and kinetic studies of low molecular weight metal complexes contribute to the better understanding of the structural and functional properties of metalloproteins and metalloenzymes. Using biomimetic metal complexes makes us possible to investigate the biological, biochemical processes that are taking place in biological systems or in living organisms. Beside this it allows the determination of the structural porperties of the active centre what is often impossible in the case of the native systems. These investigations are not only simpler and cheaper than those of the native macromolecules but provide also an opportunity for developing so called artificial enzymes by means of highly active model systems.

The aim of investigations connected to the enzyme mimetic research of metal complexes can be different. One has to distinguish the structural and the funcitonal modelling. Structural modelling of metalloenzymes means the development of model compounds that mimick the redox, magnetic, spectral and other properties of the native molecules active centre. The investigation of functinal models can help us to achieve better knowledge of the activity and working mechanism of metallonezymes. Functional modelling does not definitely pursue the exact copy of the active centre although good structural similarity often brings outstanding possibility in the enzyme mimetic activity of the complexes.

The common in modelling the active site of metalloenzymes and metalloproteines in every case that the goal is to establish an enviroment around the central metal ion which is similar to that is present in the native system. For this purpose it is possible to use synthetic (non peptide) ligands in which the donour groups are not completely identical to the donor set of the native ligand but their geometrical position provides similar structure to that can be found in the native enzyme. Another possibility is the planning of such enzymes that are

containing the same donour groups as the native ligand but in a shorter sequence. Finally it is also possible to use the same sequnece as the metal binding site of the native protein. The present theses is divided into three parts in which all the previously metioned active site modelling methods can be found.

In the first part we have investigated the iron(III) and copper(II) complexes of the salen derivative bhbdpa (N,N'-ethylenebis(salicylideneimine)) ligand (Figure 1.). The additional carboxylate group may notably alter the structure and stability of the formed complexes as compared to the corresponding species of salen and create a possibility for the formation of dinuclear units. The mononuclear complexes of bhbdpa may be of relevance to structural mimicking of the active centre of protocatechuate 3,4-dioxygenase and galactose oxydase, while the dinuclear complexes of bhbdpa provide new examples for carboxylate bridged dimetallic cores.

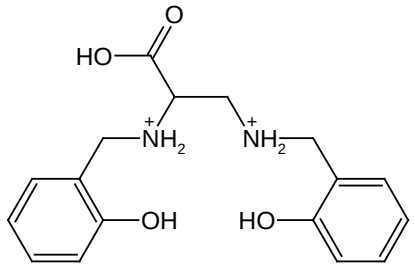
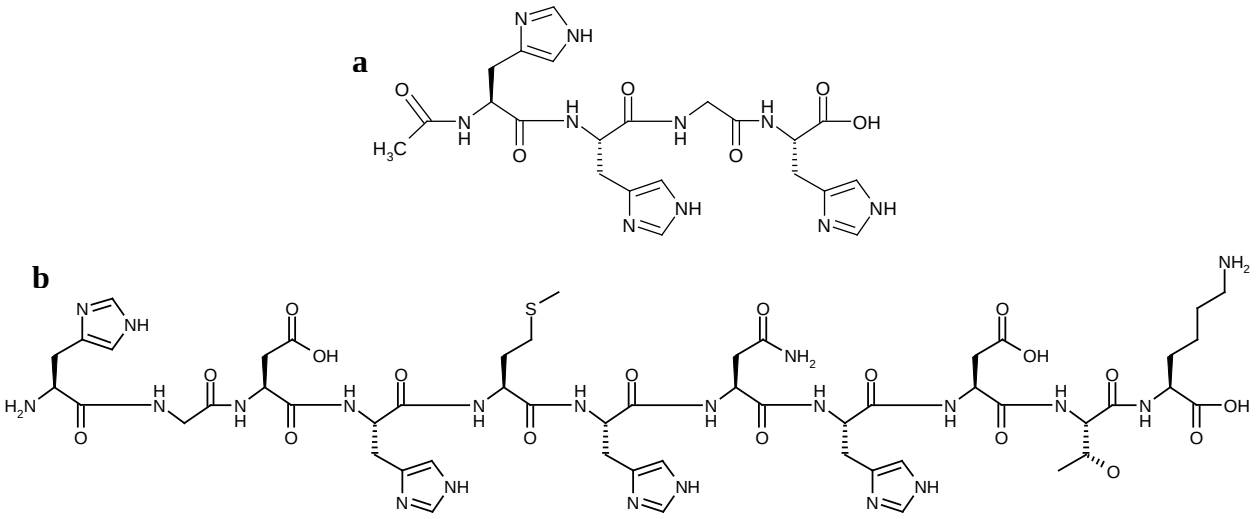


Figure 1. Structure of the bhbdpa ligand

In the second and third part our aim was the functional and structural modelling of copper(II) containing oxidase enzymes. For the mimicking of the native enzymes histidine rich active site we used peptide ligands posessing more histidine residues. We investigated the copper(II) and zinc(II) complexes of multihistidine ligands that are able to bind two or three metal ions.

With the Ac-His-His-Gly-His-OH (hhgh) tetrapeptide ligand (Figure 2. a) our intention was the establishment of a multihistidine environment around the central metal ion where, in the neutral pH-range, only imidazole donor groups are involved in the coordination. This



2. ábra A tanulmányozott hhgh (a) és hgd (b) peptidek szerkezete

system can be suitable for modelling the active centre of the above mentioned oxidase enzymes.

The undecapeptide H_2N-His-Gly-Asp-His-Met-His-Asn-His-Asp-Thr-Lys-OH (hgd) (Figure 2. b) is the N-terminal histidine rich peptide sequence corresponding to the first eleven amino acids of the protein isolated from the Cu,Zn-SOD enzyme of *H. ducrey* bacteria. Which has been previously proposed this N-terminal sequence play a copper(II) chaperoning role promoting the defence mechanism of the bacteria against superoxide radicals with this. To support the above assumption we investigated the copper(II) and zinc(II) complexes of the peptide in order to gather information about the metal binding abilities of this sequence. Beside these, on the University of Roma reconstitution experiments were carried out with the Cu-free N-deleted (the N-terminal 25 amino acids were missing) native protein in the presence of the hgd peptide. With this our goal was the clarification of the role of the investigated peptide sequence in the native enzyme.

During the whole work we laid special emphasis on the determination of the solution equilibrium, structural and isomeric properties of the formed complexes. On the basis of the species distribution and structure of the occuring complexes we carried out, under in advance stated circumstances, superoxide dismutase and pirokatechin oxidase mimicking activity measurements of the systems. We tempt to recognize connection between the solution structural properties and enzyme mimicking activity of the present complexes.

Between the above mentioned three systems biomimetic modelling as an experimental strategy provides closer connection.

EXPERIMENTAL

The stability constant and composition of the evolving species in aqueous solution were calculated on the basis of experimental data of pH-potentiometric titration using the Psequad and Superquad computer packages. The structures of these complexes were investigated by pH-dependent UV-Vis, CD, EPR and NMR spectroscopies.

In the case of hhgh and hgd ligands the potential enzyme mimicking species were tested in various enzymatic assays; their superoxide dismutase and catechol oxidase activities were studied. Beside these in cooperation with the University of Rome, we have analyzed the influence of the metal ion speciation on the kinetics of copper transfer from the undecapeptide to the active site of Cu-free N-deleted *H. ducreyi* Cu,Zn superoxide dismutase by carrying out reconstitution experiments.

NEW SCIENTIFIC RESULTS

bhbdpa systems

1. In the equimolar systems the pentadentate ligand forms very stable, differently protonated mononuclear complexes with both copper(II) and iron(III) metal ions.

2. The EPR and UV-Vis measurements in the case of [CuH_2L], [CuHL] and [CuL] species refer to the presence of microscopic complex formation complex formation pathways thus binding isomers in the solution. At two fold copper(II)ion excess the complex [Cu_2L] was detected around pH 4. Since only a weak spin-spin coupling was detected between the metal centers by EPR, probably no carboxylate bridge is present between the two {NH,PhO^-} coordinated metal ions in this dinuclear species. Above pH 5 precipitate occured in the solution.

3. In equimolar solutions in the presence of iron(III) the {NH,PhO^-,COO^-}, {2NH,2PhO^-,COO^-} and {2NH,2PhO^-,COO^-,OH^-} coordinated complexes are dominant species. At two-fold excess of iron(III) between pH 4 and 6 the μ-carboxylato-μ-hydroxo-bridged dinuclear complex [$Fe_2(OH)_3L$] is dominant. According to our knowledge bhbdpa provides a unique example for the stabilization of a carboxylate bridged diiron core in aqueous solution by a pentadentate ligand.

hhgh systems

4. In the {$3N_{im}$} and {$3N_{im}$,OH^-} coordinated [CuL] and [Cu(OH)L] complexes, formed in the neutral pH range, the ligand is coordinated to the metal ion only by its imidazole nitrogens, the amide coordinated complexes dominate only above pH 8. This offers excellent possibilities for structural/functional modelling of type II copper(II) containing metallo-enzymes.

5. In the zinc(II)-hhgh system above pH 7 precipitation occured, even in the case of ligand excess. In the [ZnL] complex probably all three imidazole rings are coordinated to the metal ion. Although the {$3N_{im}$} type coordination is one of the favored binding modes of zinc(II) in biological systems, it is not able to keep the metal ion in the solution in the present case.

6. Indeed, the species formed in the neutral pH-range are posessing high superoxide dismutase-like activity ([CuL] IC_{50} = 0,13 μM) and [Cu(OH)L] IC_{50} = 0,15 μM).

7. The [$CuH_{-1}L$] complex formed around pH 8, actually the [CuL(dtbc)] species that is formed from the [$CuH_{-1}L$], posesses outstanding activity to catalyze the oxidation of pirokatechin by dioxygen, providing the first example that copper(II)-peptide complexes are able to mimic copper containing oxidases.

hgd systems

8. Near different metal to ligand ratios in the copper(II)-hgd systems differently protonated mono-, di- and trinuclear complexes have been formed. In the neutral pH-range in equimolar solution the [CuHL] complex is the dominant species with {NH_2,$3N_{im}$} type coordination. Amide deprotonation takes place only above pH 8. In case of metal ion excess several binding isomers can be formed in the solution. In the [$Cu_2H_{-3}L$] és [$Cu_3H_{-5}L$] species, that are dominant above pH 9,5, probably {NH_2,$2N^-$,COO^-+ N_{im},$2N^-$,N_{im}} and {NH_2,$2N^-$,COO^- +N_{im},$2N^-$,N_{im}+N_{im},$2N^-$,C=O} type coordinations occur.

9. In the presence of zinc(II) the single protonated very stable [ZnHL] complex being dominant between pH 6-7, where the only proton is probably located on the non-coordinating lysine ε-amino group. Accroding to the 2D NMR measurements around the metal ion {NH_2,$3N_{im}$,S_{Met},COO^-_{Asp}} type coordination is present which has an effect on ^{1}His, ^{3}Asp, ^{4}His, ^{5}Met és ^{6}His residues of the peptide. Around pH 8 the [ZnHL(OH)] complex is the dominant. In this species eight donor groups (NH_2,$4N_{im}$,S_{Met},COO^-_{Asp},OH^-) can bind the metal ion thus probably the formation of more bindig isomers takes place that are in respectively fast exchange with each other.

10. The apparent (effective) dissociation constant (K_D) values at pH 7,4 ($K_{D,M}$ = $\Sigma[H_xL][M^{2+}_{free}]/\Sigma[MH_xL]$, $K_{D,Zn}$ = $1{,}6\times10^{-9}$ M, $K_{D,Cu}$ = $5{,}0\times10^{-12}$ M), are similar to those of the metal-trafficking proteins. This extraordinary metal ion sequestering capacity supports the already proposed copper(II) chaperoning role of the N-terminal His-rich region of *H. ducreyi* Cu,Zn SOD and may indicate similar function in the zinc(II) uptake, too.

11. According to our results the complex [CuHL] has important SOD-like activity (IC_{50} = 0,19 μM) which may indicate multifunctional role of the copper(II)-bound N-terminal His-rich domain.

PUBLICATIONS

Papers directly related to the theses

1. A. Jancsó, **Z. Paksi**, S. Mikkola, A. Rockenbauer, T. Gajda
   Iron(III)- and copper(II) complexes of an asymmetric, pentadentate salen-like ligand bearing a pendant carboxylate group
   Journal of Inorganic Biochemistry, 99, 7 (*2005) 1480-1489* IF: 2,654

2. A. Jancsó, **Z. Paksi**, N. Jakab, B. Gyurcsik, A. Rockenbauer, T. Gajda
   Solution chemical properties and catecholase-like activity of the copper(II)-Ac-His-His-Gly-His-OH system, a relevant functional model for copper containing oxidases
   Dalton Transactions, 19 (2005)3187-3194 IF: 3,012

3. **Z. Paksi**, A. Jancsó, F. Pacello, N. Nagy, A. Battistoni, T. Gajda
   Copper and zinc binding properties of the N-terminal histidine-rich sequence of *Haemophilus ducreyi* Cu,Zn superoxide dismutase
   Journal of Inorganic Biochemistry (accepted for publication) IF: 2,654

   Sum impact factor: **8,32**

Other papers

1. Zs. Árkosi, **Z. Paksi**, L. Korecz, T. Gajda, B. Henry, A. Rockenbauer
   Reinvestigation of the copper(II)–carcinine equilibrium system: “two-dimensional” EPR simulation and NMR relaxation studies for determining the formation constants and coordination modes
   Journal of Inorganic Biochemistry, 98, 12, (2004) *1995-2005* IF: 2,654

2. J. T. Kiss, K. Felföldi, **Z. Paksi,** I. Pálinkó
   Structure-forming properties of 3-furylpropenoic acid derivatives in solution and in the solid state
   Journal of Molecular Structure, 651-653, (2003) *253-258* IF: 1,495

3. E. Talabér**, Z. Paksi**, I. Pálinkó
   Intermolecular hydrogen bonding interactions between α-phenyl furylcinnamic acid stereoisomers studied by semiempirical quantum chemical method
   Journal of Molecular Structure: THEOCHEM, 620, 1(*2003*) *37-41* IF: 1,016

Conference presentations and posters

1. A. Jancsó, **Z. Paksi**, I.N. Jakab, B. Gyurcsik, A. Rockenbauer., T. Gajda
   Catecholase-like activity and solution chemical properties of the copper(II) complex of a multihistidine tetrapeptide, a functional model for copper containing oxidases
   2nd International IMBG Meeting on Metals in Biocatalysis
   Autrans (2006. September 24-27) France Poster

2. **Z. Paksi**, A. Jancsó, N. Jakab, B. Gyurcsik, A. Rockenbauer, T. Gajda
   Solution chemical properties and catecholase-like activity of the copper(II)-Ac-His-His-Gly-His-OH system, a relevant functional model for copper containing oxidases
   EUROBIC 8
   Aveiro (2006. July 2-6) Portugal Poster

3. **Paksi Z.**, Jancsó A., Rockenbauer A., Gajda T.
   Equilibrium and solution structural studies of the iron(III)- and copper(II) complexes of a novel pentadentate asymmetric salen-derivative
   XL. Komplexkémiai Kollokvium,
   Dobogókő (2005. May 18-20) Hungary Presentation

4. T. Gajda, I.N. Jakab, **Z. Paksi**, B. Gyurcsik
   Metallopeptides mimicking the structure and/or function of metalloenzymes
   International Symposium on Metals, Environment and Health
   Szklarska Poreba (2004. June 24-27) Poland Presentation

5. **Paksi Z.**, Jakab N., Gyurcsik B., Rockenbauer A., Gajda T.
   Multihisztidin peptidek réz(II) és cink (II) komplexeinek oldatkémiai vizsgálata és DNS-sel való kölcsönhatása
   XXXIX. Komplexkémiai Kollokvium
   Gárdony (2004. May 26-28) Hungary Presentation

6. I.N. Jakab, **Z. Paksi**, B. Gyurcsik, M. Győr, T. Gajda
   II-es típusú rézfehérjék szerkezeti és funkcionális modellezése multihisztidin peptidek segítségével
   XXXIX. Komplexkémiai Kollokvium
   Gárdony (2004. May 26-28) Hungary Presentation

7. **Z. Paksi**, T. Gajda, A. Jancsó
   Equilibrium and solution structural studies on the copper(II) complexes of a new, unsymmetric binucleating ligand
   Vth International Symposium: Young People and Multidisciplinary Research, Romania – Serbia & Muntenegru – Ungaria, November 6 – 7, Timisoara,
   Temesvár (2003. November 6.) Romania Presentation

8. **Paksi Z.**, Gajda T., Jancso A.
   Két új aszimmetrikus ligandum réz(II)komplexeinek egyensúlyi, oldatszerkezeti és foszfoészteráz aktivitás vizsgálata
   XXVI. Kémiai Előadói Napok (KEN)
   Szeged (2003. October 27-29) Hungary Presentation